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DESIGN AND SYNTHESIS OF HAPTEN TO INDUCE PHOSPHOLIPASE A_2-LIKE CATALYTIC ANTIBODY

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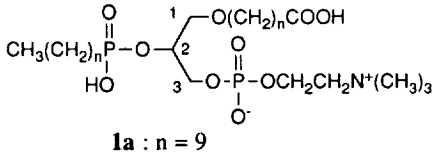
Abstract : Haptens **1a** and **1b**, transition-state analogs inducing phospholipase A_2-like catalytic antibody, were synthesized. Hapten **1a** inhibited hydrolysis of the *sn*-2 ester of phospholipid.

Since the development of catalytic antibodies by Lerner[1] and Schultz[2] in 1986, several catalytic antibodies such as those that catalyze reactions difficult to induce as conventional organic reactions have been produced[3]. Catalytic antibodies can be tailor-made for individual substrates; thus they act as very efficient catalysts. Recently they are being developed as prodrugs[4]. However, application of these catalytic antibodies has been limited primarily to production of catalysts for organic chemistry. If the ability to catalyze various biological reactions can be given to antibodies with their molecular-recognizing ability, they may become potentially useful as functional molecules that produce various physiologic activities in the biological system or in drug delivery systems.



1a : n = 9
1b : n = 15

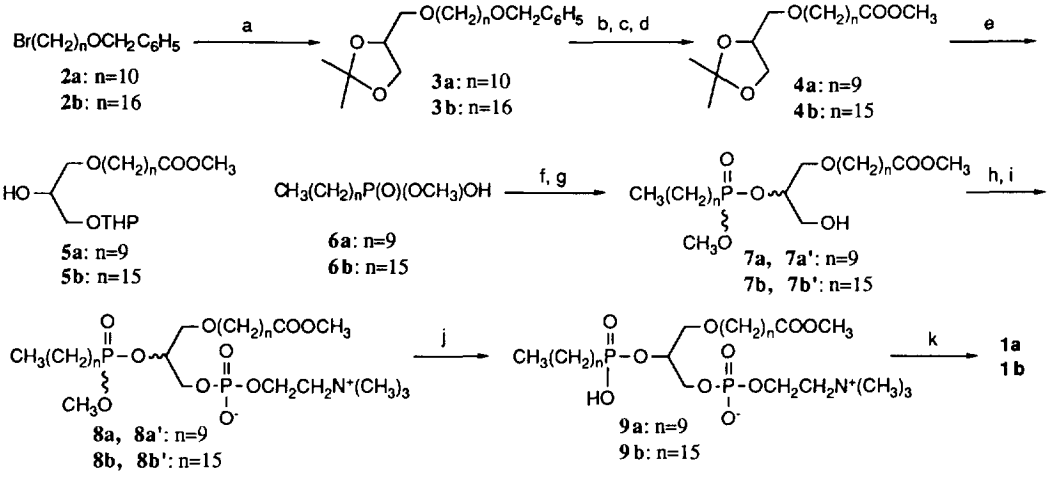
Figure 1. The structure of hapten **1a** and **1b**

Phospholipase A_2 catalyzes the hydrolysis of *sn*-2 ester of phospholipid in the cell membrane and produces lysophospholipids and fatty acids[5]. Lysophospholipids, which form spherical micelles, have the characteristic of the amphoteric surfactant and are known to cause instability of the cell membrane such as fusion and disruption depending on their concentration[6]. Antibodies with such catalytic activities are expected to be useful as functional molecules to induce membrane fusion or disruption.

In this study, to produce such catalytic antibodies and prepare artificial enzymes that selectively hydrolyze phospholipids, we designed haptens mimicking the process of hydrolysis and evaluated their synthesis.

We designed haptens **1a** and **1b** with the basic skeleton of phosphatidylcholine which exists in abundance in biological membranes. Hapten **1a** has a C10 aliphatic side chain at C_1 and C_2 that scarcely exists in phospholipids of the biological membrane and hapten **1b** has a C16 aliphatic side chain at the same positions. Position C_2 of both haptens are in the phosphonate transitional state mimicking the tetrahedral intermediate that hydrolyzes *sn*-2 ester of phospholipids. Also, an ether bond was introduced to position C_1 of the glycerol of the haptens to prevent hydrolysis by mouse esterase during antibody production, and a terminal carboxylic acid residue was introduced for binding with the carrier protein.

Haptens **1a** and **1b** were synthesized according to scheme 1. Benzyl bromide **2a** was obtained from 1,10-dibromodecane and benzylalcohol in the presence of 50 % NaOH and tetra-butylammonium hydrogen sulfate in 63.6 % yield. Compound **2b** was prepared by the Grignard reaction between 6-benzyloxyhexyl bromide and 1,10-dibromodecane in the presence of copper (I) bromide[7]. These compounds were combined with solketal to give **3a** and **3b**, followed by hydrogenolysis of the benzyl group on palladium on carbon with hydrogen and oxidation of alcohol with pyridinium dichromate, the resulting carboxylic acids were methylated with diazomethane to afford **4a** and **4b**, respectively. Compounds **4a** and **4b** were treated with camphorsulfonic acid to give the diols, followed by the protection of the primary alcohol with dihydropyran to give **5a** and **5b** in 51.1% and 33.4% yield, respectively.



Scheme 1. Synthesis of haptens **1a** and **1b**

Reagents; a) NaH, solketal, DMSO (**a**: 60.6 %; **b**: 46.5 %); b)10 % Pd-C, H_2 (**a**: EtOAc, quant.; **b**: hexane, quant.); c) PDC, DMF (**a**: 57.1 %; **b**: 79.7 %); d) CH_2N_2, Et_2O (**a**: quant.; **b**: 75.4 %); e) i) CSA, MeOH, ii) DHP, CSA, CH_2Cl_2 (**a**: 49.8 %; **b**: 33.4 %); f) i) $SOCl_2$, benzene, ii) Et_3N, $CHCl_3$ (**a**: **5a**, DMAP, 59.1 %; **b**: **5b**); g) CSA, MeOH (**a**: 83.4 %; **b**: 52.9 % from **6b**); h) $BrCH_2CH_2OP(O)Cl_2$, Et_3N, (**a**: Et_2O, 42.3 %; **b**: $CHCl_3$, 48.7 %); i) Me_3N, $CHCl_3$ (**a**: 86.7 %; **b**: 50.1 %); j) C_6H_5SH, Et_3N, (**a**: dioxane, 95.8 %; **b**: THF, 65.8 %); k) LiOH-H_2O, MeOH (**a**: 72.6 %; **b**: 89.5 %)

To introduce side chains at C_2 of **5a** and **5b** as in **1a** and **1b**, phosphonates **6a** and **6b** were prepared by the method of Dickert Jr.[8] Chlorophosphonates, prepared from **6a** and **6b** with thionylchloride, were treated with **5a** and **5b** under the presence of triethylamine in anhydrous chloroform to give the transition-state derivatives, then diastereomeric intermediates **7a** and **7a'** (1/1) were obtained from **5a** and **7b** and **7b'** (1/1) from **5b** by treatment with camphorsulfonic acid in 49.3% and 52.9% yield, respectively[9]. These diastereoisomers were converted to the target haptens **1a** and **1b** by Heyman's method[10]. Thus, compounds **7a** and **7a'** were treated with 2-bromoethyl phosphoryl dichloride in the presence of triethylamine, followed by treatment of the resulting bromides with a large excess of triethylamine to afford the twitter ionic diastereoisomer **8a** and **8a'** (1/1) in 36.7% yield. Hapten **1a**[11] could be obtained at a yield of 69.6% by demethylation of phosphonates **8a** and **8a'** and then by hydrolysis using lithium hydroxide of methylester. Compounds **7b** and **7b'** were also converted to the desired hapten **1b**[11] by the same method as above in 14.3% yield.

Since the synthetic haptens have a transition state analog that mimics the tetrahedral intermediate for hydrolysis of phosphatidylcholine, they are expected to be useful as phospholipase A_2 inhibitors[12]. The inhibitory activities of **9a** and **9b**[13] against hydrolysis of phospholipids were detected by the colorimetric assay for free fatty acids[14]. The hydrolytic activity of phospholipase A_2 decreased with the increase in the **9a** concentration, and the 50% inhibitory concentration (IC_{50}) of **9a** was 9.75 mM. The inhibitory activity of this compound was about a quarter compared with that of the single-chain choline-containing phosphonate by Gelb et al.[12] On the other hand, the inhibitory activity of **9b** could not be determined by the same method, because aggregation occurred between **9b** and phosphatidylcholine[15].

According to the results of X-ray analysis of the complexes of the phosphonate transition-state analog (inhibitor) with phospholipase A_2 obtained from cobra venom[16] and bee venom[17] reported by Sigler et al., the charges and the length of the side chain in the phosphonate transition-state analog are stabilized and recognized by the hydrophobic channel of the enzyme.

These findings suggest that compounds **1a** and **1b** are potential haptens for production of phospholipase A_2-like catalytic antibodies.

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9. Diastereomers **7a** and **7a'** (1/1) from **5a** and **7b** and **7b'** (1/1) from **5b** were used for further reaction without purification.
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11. Data for compound **1a** : ^{1}H-NMR (CD_3OD) δ : 0.89 (t, 3H, J = 6.05 Hz, -$\underline{CH_3}$), 1.29-1.31 (m, 24H, -CH_2-), 1.58 (m, 8H, -CH_2-), 2.19 (t, 2H, J = 7.73 Hz, -$\underline{CH_2}$CO-), 3.22 (s, 9H, -$N^+(\underline{CH_3})_3$), 3.45 (m, 2H, 1-$CH_2O\underline{CH_2}$-), 3.59-3.64 (m, 4H, 1-$\underline{CH_2}$O- and -$\underline{CH_2}$N-), 4.00 (m, 2H, 3-$\underline{CH_2}$O-), 4.33 (m, 3H, PO$\underline{CH_2}CH_2$- and 2-$\underline{CH}$O-). FABMS (+) *m/z* : 632 $(MH)^+$, 638 $(M+Li)^+$, 644 $(M-H+2Li)^+$.
Data for compound **1b** : ^{1}H-NMR (CD_3OD) δ : 0.89 (t, 3H, J = 7.06 Hz, -$\underline{CH_3}$), 1.28 (br. s, 48H, -CH_2-), 1.60 (m, 8H, -CH_2-), 2.14 (t, 2H, J = 8.06 Hz, -$\underline{CH_2}$COOH), 3.22 (s, 9H, -$N^+(\underline{CH_3})_3$), 3.45 (m, 2H, 1-$CH_2O\underline{CH_2}$-), 3.59-3.68 (m, 4H, 1-$\underline{CH_2}$O- and -$\underline{CH_2}$N-), 4.00 (m, 2H, 3-$\underline{CH_2}$O-), 4.33 (m, 3H, PO$\underline{CH_2}CH_2$- and 2-$\underline{CH}$O-). FABMS (+) *m/z* : 800 $(MH)^+$, 822 $(M+Na)^+$, 844 $(M-H+2Na)^+$.
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13. Colorimetric assays of methyl ester **9a** and **9b** were used instead of haptens **1a** and **1b** because of the presence of the negative charge in terminal carboxylic acid at glycerol C-1 position. **9a**: ^{1}H-NMR (CD_3OD) δ : 0.89 (t, J = 6.72 Hz, 3H, -$\underline{CH_3}$,), 1.29-1.30 (m, 24H, -CH_2-), 1.58 (m, 8H, -CH_2-), 2.30 (t, J = 7.39 Hz, 2H, -$\underline{CH_2}$CO-), 3.22 (s, 9H, -$N^+(\underline{CH_3})_3$), 3.45 (m, 2H, 1-$CH_2O\underline{CH_2}$-), 3.60-3.66 (m, 4H, 1-$\underline{CH_2}$O- and -$\underline{CH_2}$N-), 3.64 (s, 3H, $COO\underline{CH_3}$), 4.00 (m, 2H, 3-$\underline{CH_2}$O-), 4.35 (m, 3H, PO$\underline{CH_2}CH_2$- and 2-$\underline{CH}$O-). FABMS (+) *m/z* : 646 $(MH)^+$. **9b**: ^{1}H-NMR (CD_3OD) δ : 0.89 (t, 3H, J = 7.06 Hz, -$\underline{CH_3}$), 1.28 (br. s, 48H), 1.59 (m, 8H, -CH_2-), 2.30 (t, 2H, J = 7.40 Hz, -$\underline{CH_2}$CO-), 3.22 (s, 9H, -$N^+(\underline{CH_3})_3$), 3.45 (m, 2H, 1-$CH_2O\underline{CH_2}$-), 3.61 (m, 4H, 1-$\underline{CH_2}$O- and -$\underline{CH_2}$N-), 3.64 (s, 3H, -$COO\underline{CH_3}$), 4.00 (m, 2H, 3-$\underline{CH_2}$O-), 4.33 (m, 3H, PO$\underline{CH_2}CH_2$- and 2-$\underline{CH}$O-). FABMS (+)*m/z* : 814 $(MH)^+$.
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